Morphology and ontogeny of wing bud development during metamorphosis in females of the wingless bagworm moth *Epichnopterix plumella* (Denis & Schiffermüller, 1775) (Psychidae)

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Abstract. Female adults of the bagworm moths belonging to the subfamily Epichnopteriginae are vermiform in body shape, i.e., without external appendages. Larvae of the vermiform female *Eumeta variegata* (Snellen) belonging to the subfamily Oiketicinae have small wing rudiments, which do not grow but disappear during the prepupal stage. Herein we describe the morphology of wing buds in the wingless female bagworm moth *Epichnopterix plumella* (Denis & Schiffermüller), subfamily Epichnopteriginae and discusses the different developmental patterns underlying the vermiform structures in the two subfamilies. Using light microscopy, we show that the structure of the wing discs in the last larval instar is similar in both sexes. Scanning electron microscopy observations also show that the pupal wing cases of the female are much smaller than those of the male, and that female adults completely lack wing rudiments. We show that the three main wing tracheae, i.e., Radius, Media and Cubitus, in the female pupa extend nearly to the wing margin. Hence, our studies demonstrate that the loss of wings in the vermiform morph of the female adult in these exemplars of the subfamilies Oiketicinae and Epichnopteriginae is achieved through different developmental processes.

Introduction

Brachypterous (reduced wings) or apterous (no wings) adults are a recurrent phenomenon among several orders of holometabolous insects and are secondarily derived from a winged morph in their evolutionary histories. This secondary loss of wings in insects has typically been interpreted as an evolutionary adaptation to increase fecundity (Roff 1990, Wagner & Liebherr 1992). In Lepidoptera, female-specific wing degeneration occurs in many groups; that is, brachypterous or apterous forms are known in 25 of about 120 families (Sattler 1991). Globally, the family Psychidae comprises nearly 1000 species (Davis & Robinson 1999). The larvae of these moths construct larval cases from dead leaves, small twigs and/or other foreign bodies; hence, the members of this family are called bagworm moths. Adult females are usually wingless, but the females of the presumably earliest lineages have normal wings. Bagworm moths may have lost their wings during evolution due to their case-dwelling life style. We have reported the ontogenetic study of wing degeneration in several species of Japanese wingless bagworm moths. These degeneration processes were caused by apoptosis during the larval-pupal

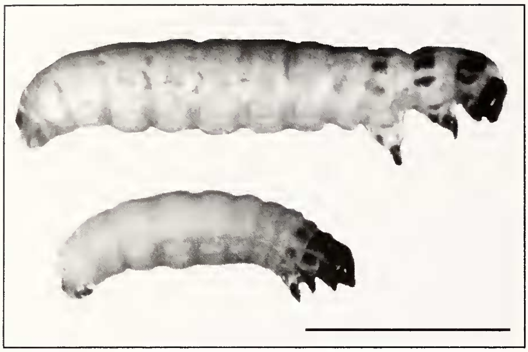

Fig. 1. Fully-grown female (upper) and male (lower) larvae of *Epichnopterix plumella*. The body length is about 12 mm in females and 7 mm in males. Scale bar: 5 mm.

development or pupal-adult development (Niitsu & Kobayashi 2008; Niitsu et al. 2008). However, the evolutionary and developmental processes underlying these dramatic changes in phenotype are still not fully understood.

In some derived groups, such as the subfamilies Oiketicinae and Epichnopteriginae, the female adults are vermiform, i.e., completely lacking wings and other appendages (Hättenschwiler 1985). The pupa of the wingless female bagworm moth *Epichnopterix plumella* (Denis & Schiffermüller, 1775) is illustrated by Patočka and Turčáni (2005). According to their study, the females of *E. plumella* are unique among the examined taxa in that the pupa possesses rudimentary wing cases while the adult lacks wings. Our knowledge of the morphology and ontogeny of the wing buds of this group is still poor, especially regarding histological details. This developmental pattern, i.e., the female pupa possessing wing cases but adult being apterous, has not been found in any other species of wingless bagworm moths. Therefore, to understand the evolution of wing reduction in the family, it is necessary to clarify the unique process of degeneration in wingless female epichnopterigines.

In this paper, we describe the postembryonic development of the wing bud in *E. plumella* and compare this in relation to the pattern of wing reduction in several other taxa of wingless bagworm moths. This is the first morphological and histological study of the process of wing reduction in Epichnopteriginae.

Material and Methods

Collection of Larvae. Several final instar larvae of *E. plumella* were collected in the United Kingdom (Hainault Forest, Essex) in April of 2009 and were kept at room temperature (about 20°C) for a few weeks. The mature larvae of *E. plumella* are easily sexed on the basis of size (Fig. 1). In order to check whether a last instar larva was a prepupa, its larval case was opened with scissors, following the criteria of Niitsu (2003). Both sexes spend about 5 days as a prepupa, during which the gut is purged of ingested food prior to pupation. At least three examples of female last instar larvae, prepupae, and pupae were fixed. Any males of last instar larvae were also fixed.

Histological Observations. For histological observations, the wing discs of larvae and pupal wings were removed and fixed in Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde), then buffered with 0.1M HCl-sodium cacodylate. They were then treated with 2% osmium tetroxide, dehydrated in a series of ethanol and propylene oxide and embedded in Epon 812 (TAAB). Semi-thin sections (1 μm thick) of the wing buds were prepared on a rotary microtome and mounted on microscope slides before

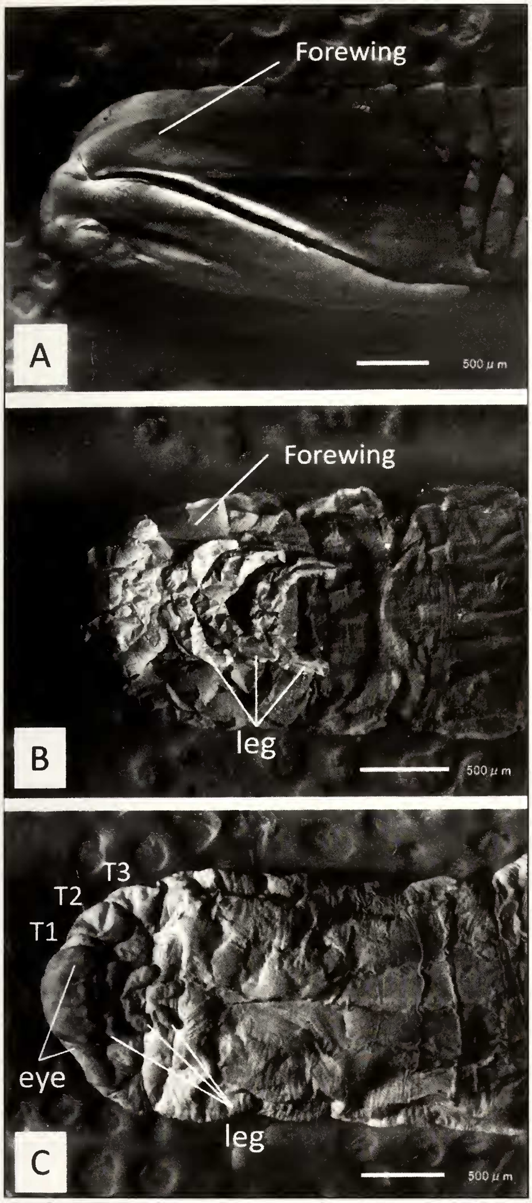

Fig. 2. Scanning electron micrographs of a male (**A**) and female pupa (**B**) and a wingless female adult (**C**) of *Epichnopterix plumella*. Anterior is left. Scale bars: 500 µm.

staining with Azur B. Slide-mounted tissues were observed using a NIKON OPTIOHOT microscope.

Scanning Electron Microscopy. Scanning electron microscopy (SEM) was used to examine the structure of pupae of both sexes, and the external structures of female adults. Samples were fixed in Karnovsky's fixtative, then treated with 2% osmium tetroxide and dehydrated in a graded series of ethanol (up to 100%). After critical-point drying, scanning electron micrographs were taken using a JSM-6510LV electron microscope (JEOL, JAPAN).

Results

Pupae of Both Sexes. The body length of the examined male pupa is 3.8 mm (Fig. 2A), while the female was 4.1 mm (Fig. 2B). Female pupal wing cases are extremely reduced, their length being about 0.5 mm. In contrast, the pupal wing cases of the males are well developed (Fig. 2A), the forewing length being about 3.0 mm.

Adult Females. The body length of the female adult *E. plumella* examine in the SEM (Fig. 2C) is 4.9 mm. They possess a pair of rudimentary eyes and have three pairs of small non-segmented legs. The head is strongly reduced, but the thoracic segments are easily recognized. Wings are completely absent.

Wing Imaginal Discs. To study female wing reduction in *E. plumella*, the development of wing discs during the last larval instar in the female was observed by dissection. In the middle stage of the last-larval instar, the wing imaginal discs were oval in shape for both sexes (Figs 3A, B). The development of the wing discs progressed in the same way for both sexes until the last larval stage when the female wing disc was evaginated during the prepupal stage. Just before pupation of the female larva, proliferation of the wing buds stopped and the wing epithelial cells secreted a new pupal

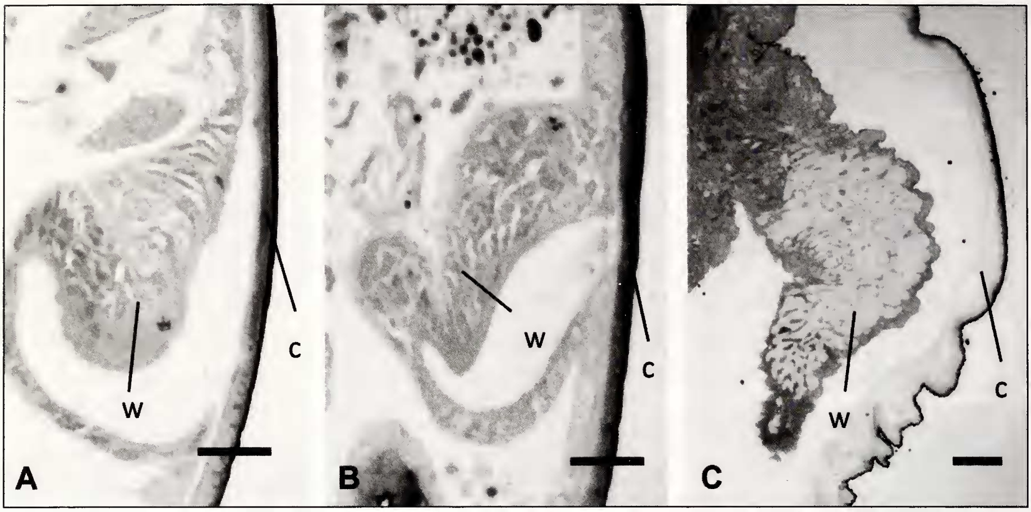

Fig. 3. Cross sections of the wing disc of *Epichnopterix plumella*. Sectioned (**A**) half way through the last larval instar of male; (**B**) half way through the last larval instar of female; (**C**) late female prepupa of the last instar (note the evaginated wing bud and the lamination of the new pupal cuticle). w-wing bud; c-cuticle. Scale bars: 20 μm (A, B) and 50 μm (C).

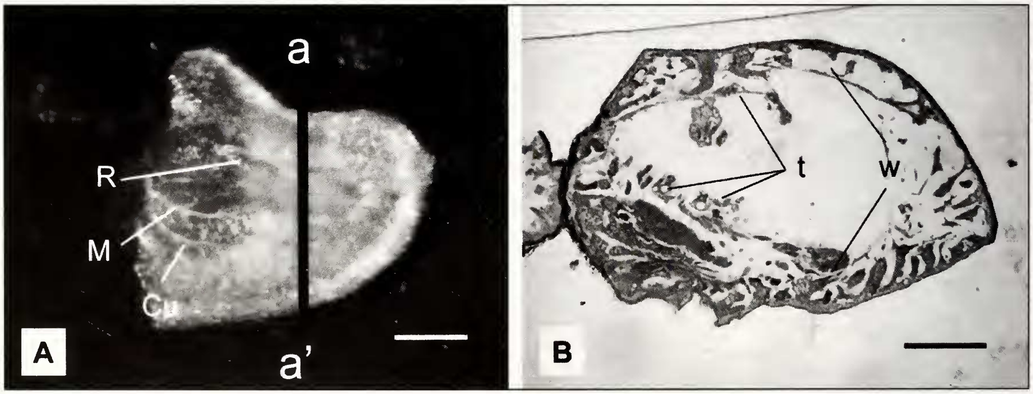

Fig. 4. Female pupal wings of *Epichnopterix plumella* just after pupation. (**A**) Female forewing dissected from pupa. (**B**) Pupal wings of female with a much simpler tracheal system than those of males. a-a' levels of the section depicted in Fig B. R-Radius; M-Media; Cu-Cubitus; t, trachea; w, pupal wing epithelium. Scale bars: 100 μm (A) and 50 μm (B).

cuticle (Fig. 3C). In this study, male wing buds were not examined. However, the male pupal wings demonstrate that the wing buds of males are larger than those of females.

Pupal Wings of Females. In the female pupa of *E. plumella*, the three main wing tracheae, i.e., those pertaining to R(adius), M(edia) and Cu(bitus), extended nearly to the margin of the wings (Fig. 4A). During the pupal stage (day 0), the wing epithelia of females are attached to the pupal cuticle (Fig. 4B). On day 2 (48h after pupation), the female wing epithelia had already retracted completely from the pupal wing case (not illustrated); hence the moth is now in the pharate adult stage.

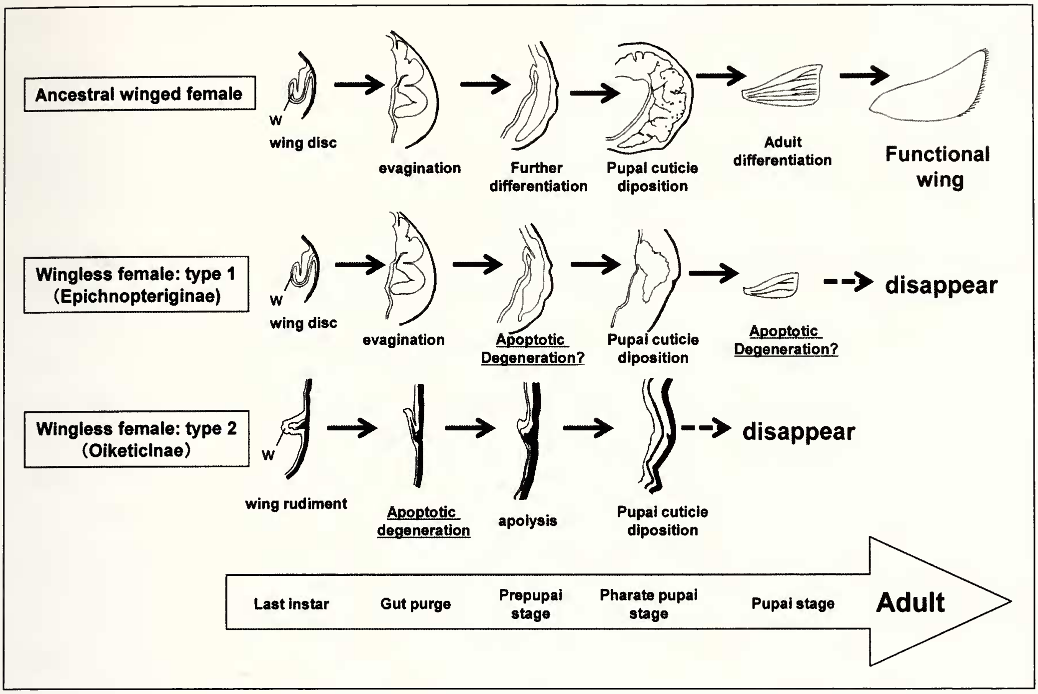

Fig. 5. Schematic diagram of differential wing formation and degeneration in Epichnopteriginae and Oiketicinae. w-wing discs or wing rudiments. The different methods of wing reduction between the two subfamilies are indicated.

Discussion

The present study demonstrates that females of *E. plumella* possess wing discs during larval-pupal and pupal-adult development. Although we could not confirm whether there is an apoptotic event during female wing development, our results suggest that female-specific wing degeneration occurs in two steps, i.e., in both the larval and pupal stages (Fig. 5). Niitsu & Kobayashi (2008) similarly reported that in females of three species of wingless but fully-legged bagworm moths, *Taleporia trichopterella* (Saigusa, 1961), *Bacotia sakabei* (Seino, 1981) and *Proutia* sp., wing buds were reduced due to apoptosis in the same two steps, i.e., during the larval and pupal stages. This study suggests that the female degeneration pattern is similar in all before-mentioned taxa.

Wing degeneration has been reported in females of several other psychid moths. In *Eumeta variegata* (Snellen, 1879), subfamily Oiketicinae, the external morphology of the female pupae is entirely different from the male: the female pupa is "vermiform" without wing cases and the female adult is apterous; the vestigial wing rudiments do not grow but disappear during the prepupal stage (Niitsu 2003). In the female of another oiketicine psychid *Pachytelia unicolor* (Hufnagel, 1766), the development of the wing discs is suppressed in an early larval instar (Fedotov 1939). It has been hypothesized that the vermiform state of the females in Psychidae was the most specialized (i.e., derived) condition in the evolution in the family and that wingless females evolved from winged females (Saigusa 1962, Yen et al. 2004). Our data on the presence of larval or

pupal wing buds in female wingless psychids is also compatible with the hypothesis that taxa with wingless females evolved from taxa with winged ones. We here classify the pattern of female wing reduction in several bagworm moths into two types (Fig. 5): 1) wingless female type 1, in which female-specific wing degeneration occurs in two steps, i.e. in the larval and pupal stages (subfamily Epichnopteriginae); 2) wingless female type 2, in which the minute wing rudiment does not grow and disappears during larval-pupal development (subfamily Oiketicinae).

In Lepidoptera, females with reduced wings are known in Geometridae, Noctuidae, Psychidae and several other families, and there have been some studies of these patterns of female-specific wing reduction (Common 1970, Hackman 1966, Hamilton 1978, Heppner 1991, Matsuda 1979, Sattler 1991). In the lymantriine *Orgyia leucostigma* (Smith, 1797), female wing discs are formed initially but are later destroyed by apoptosis during pupal-adult development (Nardi et al. 1991). In the congener *O. dubia* (Tauscher, 1806), the female pupa completely lacks pupal wing cases and the female adult is vermiform and apterous (Mostafa & Laila 1969), but nothing is known about the developmental process of wing reduction in this species. In yet another *Orgyia* species, *O. thyellina* Butler, 1881, the female wings show seasonal dimorphism. The autumn females have short wings whereas the summer females have normal wings (Sato 1977). In the winter moth *Nyssiodes lefuarius* (Erschoff, 1872) (Geometridae), female wing discs are fully developed but are destroyed suddenly by apoptosis in the late pupal stage (Niitsu 2001).

The ecdysone hormone, ecdysteroid, plays an important role in induction of female-specific wing degeneration among flightless lepidopterans (Lobbia et al. 2003). Niitsu et al. (2008) clarified that female-specific wing degeneration in *E. variegata* is triggered directly by ecdysteroids in female wing rudiment cultured in vitro. They have recently reported that female wing rudiment of *E. variegata* lose the ability for cell proliferation in response to the stimulus of 20-hydroxyecdysone (Niitsu et al. 2011). However, the molecular mechanisms causing female-specific winglessness in this species are not yet fully understood.

Conclusions

We have here demonstrated that the vermiform morph of the female adults in examined representatives of Oiketicinae and Epichnopteriginae have different developmental backgrounds, i.e., the evolutionary wing loss has been achieved in different ways. Whether these similar (vermiform) morphs, arising via two different pathways of wing loss, occurred as a result of parallel evolution, or whether they represent steps in a single transformation series can be fruitfully discussed only on the basis of a robust phylogeny of the family. Work towards this goal, together with further studies of developmental biology and comparative histology, should improve our understanding of the evolution of the female-specific wingless condition in Psychidae.

Acknowledgments

This work was supported by a grant of the Fujiwara Natural History Foundation in 2009.

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